102. The method of claim 90, wherein the hybridization conditions are selected from the group consisting of very low, low, low-medium, medium, medium-high, high, and very high stringency conditions.

REMARKS

Claims 1-5, 11-13, 19, 20, 26-28, 34, 40-42, 62, 64, and 65 have been canceled. New claims 90-102 have been added and are pending in the present application.

It is respectfully submitted that the present amendment presents no new issues or new matter and places this case in condition for allowance. Reconsideration of the application in view of the above amendments and the following remarks is requested

I. The Restriction Requirement

The Office Action imposed restriction and election requirements. Specifically, Applicants were requested to elect between the following groups:

Group I: Claims 1-5, 11-13, 19, 20, 26-28, 34, and 40, drawn to a method of differential gene expression analysis, classified in class 435, subclass 6

Group II: Claims 41, 42, and 62 drawn to computer readable media comprising data, classified in class 365, subclass 94.

Group III: Claims 64 and 65, drawn to arrays of polynucleotides, classified in class 536, subclass 24.32.

As provided therein, Applicants provisionally elected with traverse the claims of Group I. Applicants confirm the election of claims 1, 2, 11, 19, 20, 34 and 40 and the species *Aspergillus oryzae* and corresponding EST combination of SEQ ID NOS:4377- 7401.

Applicants reserve the right to file continuing applications directed to the non-elected subject matter.

II. The Rejection of Claim 20 under 35 U.S.C. § 101

Claim 20 stands rejected under 35 U.S.C. § 101 on the ground that the claimed invention lacks patentable utility. The Office Action states:

> The claimed combination of nucleic acids is not supported by a substantial utility because no substantial utility has been established for the claimed subject matter. The disclosed utility is to use an array of *Aspergillus oryzae* ESTs to determine expression profiles that are correlated with different types of cells or different growth states of cells. No evidence has been disclosed that the elected SEQ ID NOS allow for determination of the state or type of cell that is assayed by the claimed method of using an array of *Aspergillus oryzae* ESTs. Further research is required to determine whether the claimed method utilizes ESTs that allow for useful discrimination between cell types or cell states. The research required to establish the utility of the claimed method is not consistent with a substantial utility. Identifying and studying the properties of an array of ESTs does not define a "real world" context or use. Neither the specification as filed nor any art of record discloses or suggests any property or activity for the array of ESTs such that another non-asserted

utility would be well established for the compounds.

This rejection is respectfully traversed.

Applicants disagree with the Office Action's statement that the "claimed combination of nucleic acids is not supported by a substantial utility" because "[n]o evidence has been disclosed that the elected SEQ ID NOS allow for determination of the state or type of cell that is assayed by the claimed method of using an array of *Aspergillus oryzae* ESTs."

Filamentous fungi are increasingly being used as host microorganisms for the industrial production of enzymes, antibiotics, biochemicals, fermented foods, and other proteins whether endogenous or heterogenous to the microorganisms. There is a need in the art to provide methods for monitoring the global expression of genes from filamentous fungal cells to improve the production potential of these microorganisms. Applicants provide detailed protocols for monitoring differential expression of a plurality of genes in a filamentous fungus of interest. These protocols include a set of *Aspergillus oryzae* EST sequences with their nucleic acid sequences and function identified (see Table 3; page 4, line 28, to page 7, line 7); methods and instruments for forming microarrays on the surface of a solid support (see page 7, line 9, to page 11, line 3); preparation of nucleic acid probes from filamentous fungi and their labeling with reporters, e.g., Cy3 and Cy5 (see page 11, line 5, to page 13, line 9); hybridization of the probes with the arrays containing the *Aspergillus oryzae* ESTs (see page 13, line 11, to page 24, line 24; and methods of detection and data analysis (see page 14, line 26, to page 15, line 21).

Based on Applicants' disclosure, one of ordinary skill in the art would be able to set-up and use the methods of the present invention to monitor global expression of a plurality of genes from a filamentous fungal cell with respect to a particular phenotype such as improved secretion or production of a protein or compound, reduced or no secretion or production of a protein or compound, improved or reduced expression of a gene or pathway, desirable morphology, an altered growth rate under desired conditions, relief of over-expression mediated growth inhibition, or tolerance to low oxygen conditions; to discover new genes; to identify possible functions of unknown open reading frames; and to monitor gene copy number variation and stability. For example, the global view of changes in expression of genes may be used to provide a picture of the way in which filamentous fungal cells adapt to changes in culture conditions, environmental stress, or other physiological provocation. Applicants also indicate that other possibilities for monitoring global expression include spore formation/germination, recombination, metabolic or catabolic pathway engineering.

The methods of the present invention are particularly advantageous because one spot on an array equals one gene or open reading frame; extensive follow-up characterization is unnecessary since sequence information is available as shown in Table 3 of the specification, and EST microarrays can be organized based on function of the gene products. Applicants provide in Table 3 annotated identification of the open reading frames of the *Aspergillus oryzae* ESTs.

Example 16 of the specification is an example of monitoring multiple changes in expression of *Fusarium venenatum* genes to specifically identify those genes whose expression (a) increases by a factor of approximately two (b) remains the same, or (c) decreases by a factor of approximately two in response to the presence of maltose as a sole

carbon source. *Fusarium venenatum* strain CC1-3 was grown in Vogel's minimal medium with either 2% glucose or 2% maltose as the sole carbon source. After 2 days growth at 28°C, total RNA and mRNA pools were purified from each culture. PolyA-selected mRNA was used as a template to prepare fluorescently labeled probes for hybridization. In this experiment, the probes from glucose-grown cells were labeled with Cy3 and the probes from maltose-grown cells were labeled with Cy5. The probes were combined and hybridized with the 1152 EST targets on the microarray. After hybridization and washing, the microarrays were scanned (see Example 15), and the images analyzed using ScanAlyze software (see Example 15) to determine the relative ratios of red and green fluorescence in each spot on the arrays. A number of genes satisfying the above criteria were readily identified as shown in Table 5. A person of ordinary skill in the art would be able to perform the same experiment on *Aspergillus oryzae* using selected ESTs of SEQ ID NOs. 4377-7401 by following the detailed protocols provided by the Applicants

The Office Action also states "[i]dentifying and studying the properties of an array of ESTs does not define a 'real world' context or use." Applicants respectfully disagree with this statement because the present invention is not directed to identifying and studying the properties of an array of ESTs, but rather to use an array of *Aspergillus oryzae* ESTs to determine expression profiles of a plurality of genes that correlate, for example, with adaptation to changes in culture conditions, environmental stress, or other physiological provocation of a filamentous fungal cell of interest.

Applicants assert, therefore, that the claimed combination of nucleic acids is supported by a substantial patentable utility.

For the foregoing reasons, Applicants submit that the rejection under 35 U.S.C. § 101 has been overcome and respectfully request reconsideration and withdrawal of the rejection.

III. The Rejection of Claim 20 under 35 U.S.C. 112, First Paragraph

Claim 20 is rejected under 35 U.S.C. 112, first paragraph, on the ground that one skilled in the art would not know how to use the claimed invention since it is not supported by a substantial utility or a well established utility, as described in Section II. This rejection is respectfully traversed.

Based on Applicants' arguments in Section II, Applicants assert that one skilled in the art would know how to use the claimed invention because it is supported by a substantial utility.

For the foregoing reason, Applicants submit that the rejection under 35 U.S.C. § 112 has been overcome and respectfully request reconsideration and withdrawal of the rejection.

iV. The Rejection of Claim 20 under 35 U.S.C. 112, First Paragraph

Claim 20 is rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. The Office Action states

> The specification discloses SEQ ID NO: 4377-7401. SEQ ID NO: 4377-7401 meet the written description provisions of 35 USC 112, first paragraph. However, because it is not apparent that

SEQ ID NO: 4377-7401 comprises a complete open reading frame, claim 20 is directed to encompass gene sequences and complete cDNA sequences due to the recitation of the phrase "and nucleic acid sequences having at least 90% homology to SEQ ID NOS: 4317-7401." The claims further encompass sequences that hybridize or are similar to SEQ ID NO: 4377-7401, corresponding sequences from other species, mutated sequences, allelic variants, splice variants, sequences that have a recited degree of identity (similarity, homology), and so forth. None of these sequences meet the written description provision of 35 U.S.C 112, first paragraph.

This rejection is respectfully traversed.

The Office Action states that while SEQ ID NOs: 4377-7401 meet the written description provisions of 35 U.S.C. 112, first paragraph, sequences that have a recited degree of homology do not meet the written description requirement. Applicants detail on page 7, lines 2-7, of the specification, that the degree of homology between two nucleic acid sequences is determined by the Wilbur-Lipman method (Wilbur and Lipman, 1983, *Proceedings of the National Academy of Science USA* 80: 726-730). Applicants further describe the use of SEQ ID NOs: 4377-7401 in conducting hybridization to identify genes from other strains that are closely related or essentially identical to SEQ ID NOs: 4377-7401. Once a gene is isolated and its sequence determined, the sequence can then be compared to the corresponding sequence of SEQ ID NOs: 4377-7401 to ascertain whether it falls within the scope of the instant claims. These methods are highly predictable and do not require undue experimentation. Consequently, Applicants submit that the information disclosed in the specification combined with the knowledge of the art provides sufficient guidance to one of ordinary skill in the art to isolate nucleic acids from other strains having at least 90% homology to SEQ ID NOS: 4317-7401.

Claims limited to the nucleic acid sequences of SEQ ID NOs: 4377-7401 would not adequately protect the inventors. One of ordinary skill in the art could make, for example, one or more codon changes in the sequences of SEQ ID NOs: 4377-7401 to encode conservative amino acids. Such conservative amino acid substitutions are, for example, within the group of basic amino acids (arginine, lysine and histidine), acidic amino acids (glutamic acid and aspartic acid), polar amino acids (glutamine and asparagine), hydrophobic amino acids (leucine, isoleucine and valine), aromatic amino acids (phenylalanine, tryptophan and tyrosine), and small amino acids (glycine, alanine, serine, threonine and methionine). Moreover, other codon changes in SEQ ID NOs: 4377-7401 of a minor nature could also be made, naturally or recombinantly, that do not change the inherent properties of the proteins encoded by SEQ ID NOs: 4377-7401. Thus, one skilled in the art could make such a conservative change by substitution of a different codon and thereby circumvent the literal scope of Applicants' patent rights.

For the foregoing reasons, Applicants submit that the new claims overcome the rejections under 35 U.S.C. § 112. Applicants respectfully request reconsideration and withdrawal of the rejection.

V. The Rejection of Claims 1, 2, 11, 19, 20, 34, and 40 under 35 U.S.C. § 112, Second Paragraph

Claims 1, 2, 11, 19, 20, 34, and 40 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for recitation in claim 1 part (b)(i) of the phrase "the ESTs ... produce a distinct first fluorescence emission color" and further in part (b)(ii) of the phrase "the ESTs in the array ... produce a distinct combined fluorescence emission color"

because it is not clear from the claims whether the fluorescence is produced by the fluorescence-labeled nucleic acids or by the ESTs. The rejection would be overcome by amending claim I to recite in claim I part (b) (i) the phrase "the fluorescence-labeled nucleic acids obtained from either the first or the one or more second filamentous fungal cells that are hybridized to the ESTs in the array produce a distinct first fluorescence emission color" and further in part (b) (ii) the phrase "the fluorescence-labeled nucleic acids obtained from both the first and one or more second filamentous fungal cells that are hybridized to the ESTs in the array produce a distinct combined fluorescence emission color."

Applicants have cancelled claims 1, 2, 11, 19, 20, 34, and 40, but the language suggested by the Examiner has been taken into account in the new claims which now recite "(i) the fluorescence-labeled nucleic acids obtained from the first filamentous fungal cell that are hybridized to the ESTs in the array produce a distinct first fluorescence emission color and the fluorescence-labeled nucleic acids obtained from the one or more second filamentous fungal cells that are hybridized to the ESTs in the array produce a distinct second fluorescence emission color, and (ii) the fluorescence-labeled nucleic acids obtained from both the first and the one or more second filamentous fungal cells that are hybridized to the ESTs in the array produce a distinct combined fluorescence emission color."

For the foregoing reason, Applicants submit that the new claims overcome the rejections under 35 U.S.C. § 112. Applicants respectfully request reconsideration and withdrawal of the rejection.

VI. The Rejection of Claim 20 under 35 U.S.C. § 103

Claims 20 stands rejected under 35 U.S.C. § 103(a) as being unpatentable over DeRisi *et al.* in view *of* Machida *et al.* in view of Minetoki *et al.* in view of Hata *et al.* The Office Action states:

> It would have been obvious to a person of ordinary skill in the art at the time the invention was made to modify the method of DeRisi *et al.* by use of probes functionally and structurally equivalent to ESTs that were derived from *Aspergillus oryzae* genes as shown in Machida *et al.*, Minetoki *et al.*, and Rata *et al.* because Machida *et al.*, Minetoki *et al.*, and Rata *et al.* show the utility of using such probes as a research tool to examine transcription of multiple *Aspergillus oryzae* genes.

This rejection is respectfully traversed.

The Examiner has the initial burden of establishing a *prima facie* case of obviousness. A finding of obviousness under §103 requires a determination of the scope and content of the prior art, the differences between the claimed invention and the prior art, the level of ordinary skill in the art, and whether the differences are such that the claimed subject matter as a whole would have been obvious to one of ordinary skill in the art at the time the invention was made. *Graham v. Deere*, 383 US 1 (1966). Obviousness cannot be established by combining the teachings of the prior art to produce the claimed invention, absent some teaching or suggestion that the combination be made. *In re Stencel*, 828 F2d 751, 4 USPQ2d 1071 (Fed. Cir. 1987).

DeRisi *et al.* disclose the use of a DNA microarrays containing virtually every gene of *Saccharomyces cerevisiae* to investigate on a genomic scale the termporal program of gene expression accompanying the metabolic shift from fermentation to respiration.

Michida *et al.* disclose the molecular cloning of a cDNA encoding enolase from *Aspergillus oryzae* and show the steady state levels of *Aspergillus oryzae* enolase mRNA after growth on different carbon sources.

Minetoki *et al.* disclose the nucleotide sequence and expression of an *Aspergilllus oryzae* alpha-glucosidase-encoding gene which was induced when maltose was provided as a carbon source, but expression was not induced by glucose.

Hata *et al.* disclose two functional elements of the promoter region of the *Aspergillus oryzae glaA* gene encoding glucoamylase, where both elements are essential for directing high-level expression in the presence of maltose.

However, DeRisi *et al.*, Michida *et al.*, Minetoki *et al.*, and Hata *et al.*, alone or in combination, do not teach or suggest methods for monitoring differential expression of a plurality of genes in a first filamentous fungal cell relative to expression of the same or very similar genes in one or more second filamentous fungal cells using a DNA microarray of *Aspergillus oryzae* ESTs of SEQ ID NOs. 4377-7401, as claimed in the instant invention.

Applicants assert that it is improper to combine the cited references. For prior art references to be combined to render obvious a subsequent invention under Section 103, there must be something in the prior art as a whole which suggests the desirability, and thus the obviousness, of making the combination. The Office Action suggests that it would have been obvious to a person of ordinary skill in the art at the time the invention was made to modify the method of DeRisi *et al.* by use of probes functionally and structurally equivalent to ESTs derived from the *Aspergillus oryzae* genes disclosed by Machida *et al.*, Minetoki *et al.*, and Rata *et al.*, which show the utility of using such genes as probes to examine transcription of multiple *Aspergillus oryzae* genes. But, obviousness cannot be established by combining the teachings of the prior art to produce the claimed invention, absent some teaching or suggestion supporting the combination. None of the cited references teaches or suggests the *Aspergillus oryzae* ESTs of SEQ ID NOs. 4377-7401. Machida *et al.*, Minetoki *et al.*, and Rata *et al.* disclose a total of three full-length genes from *Aspergillus oryzae*, but Applicants respectfully point out that three genes are insufficient to prepare a DNA micorarray for monitoring differential expression of a plurality of genes in filamentous fungal cells, as claimed in the instant invention. Until Applicants' isolation and charcterization of the *Aspergillus oryzae* ESTs of SEQ ID NOs. 4377-7401 and their incorporation into a DNA microarray for monitoring differential expression of a plurality of genes in filamentous fungal cells, the possibility of monitoring such differential expression was not possible. Moreover, none of the cited references teaches or suggests the use of the *Aspergillus oryzae* ESTs of SEQ ID NOs. 4377-7401 in a DNA microarray for monitoring differential expression in filamentous fungi.

Applicants submit that hindsight reconstruction is involved in the Office Action's arguments. For prior art references to be combined to render obvious a subsequent invention under Section 103, there must be something in the prior art as a whole which suggests the desirability, and thus the obviousness, of making the combination. *Uniroyal v. Rudkin-Wiley*, 5 USPQ2d 1434, 1438 (Fed. Cir. 1988). It is impermissible to use the claims as a framework from which to pick and choose among individual references to recreate the claimed invention. *In re Fine*, 5 USPQ2d 1596, 1600

(Fed. Cir. 1988). The argument presented in the Office Action may be an "obvious-to-try" argument, but such an argument is inadequate to render the claimed invention obvious without some teaching in the prior art which gives a reasonable expectation of success in achieving the goal of the methods of the claimed invention.

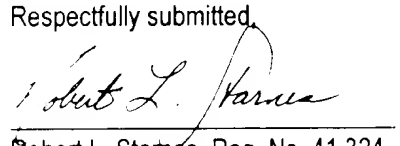
Applicants assert, therefore, that there is no motivation to combine the references to arrive at the instant invention because the cited references, alone or in combination, do not teach or suggest the methods of the present invention.

For the foregoing reasons, Applicants submit that the claims overcome this rejection under 35 U.S.C. § 103(a). Applicants respectfully request reconsideration and withdrawal of the rejection.

VII. Conclusion

In view of the above, it is respectfully submitted that all claims are in condition for allowance. Early action to that end is respectfully requested. The Examiner is hereby invited to contact the undersigned by telephone if there are any questions concerning this amendment or application.

Respectfully submitted,



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